Research Use Only

DNA/RNA Extraction Kit

User Manual

Manufacturer:

GeneReach Biotechnology Corporation

TEL: 886-4-2463-9869 Email: sales@genereach.com

No. 19, Keyuan 2nd Rd., Central Taiwan Science Park, Taichung City 407, Taiwan

Website: www.tacomag.com

2011/05

Content

Symbols **1**

Kit Components **2**

A. Reagents 2

B. Plate & Sleeve 3

Storage & Shipping **3**

Equipment and Reagents to Be Supplied by Users **4**

Introduction **5**

Intended Use **5**

Important Notes **6**

Nucleic Acid Extraction Procedure **7**

A. Use of **taco™** Sticker 7

B. Protocol 8

Product Use Limitations **12**

Trouble Shooting **13**

Appendix I **17**

Sample Preparation 17

Appendix II 18

A. Storage of Nucleic Acids 18

B. Quantification of Nucleic Acids 18

C. Purity of Nucleic Acids 19

Symbols

Date of manufacturing

Manufacturer

Lot number

Catalogue number

Do not reuse

Kit Components

A. Reagents

taco™ DNA/RNA Extraction Kit Cat. No.: atc-d/rna Number of reactions: 320		
Reagent Name	Volume	Quantity
Magnetic Bead	18 ml	1 bottle
Lysis Buffer	180 ml	1 bottle
Washing Buffer A[1]	135 ml	2 bottles
Washing Buffer B[2]	40 ml	2 bottles
Eluting Buffer	55 ml	1 bottle
User Manual		1 copy

*Treat all reagents as potential irritants.

[1] **Add 135 ml 95% ethanol to Washing Buffer A before use.**

Mark the label of buffer bottle after the addition of ethanol.

[2] **Add 230 ml 95% ethanol to Washing Buffer B before use.**

Mark the label of buffer bottle after the addition of ethanol.

B. Plate & Sleeve (For single use)

Product Name	Amount (pcs)	Cat. No.
96-Well Extraction Plate	20	atcp
Mixing Sleeve	40	
taco™ Sticker	1	

***Do not reuse the Plate & Sleeve**

Storage & Shipping

All reagents should be stored well sealed and kept dry at room temperature to the expiration date labeled on the box. Deliver all reagents at room temperature if necessary.

Expiration dates are stated on the box and on each single component of the kit. Do not use any component of the kit beyond the expiration date. Any deviation from the instruction could influence kit performance and must be validated by the users.

Equipment and Reagents to Be Supplied by Users

- **taco™** Nucleic Acid Automatic Extraction System (**taco™**)
- Step pipette (optional)
- Disposable gloves
- Micro-centrifuge tubes
- Micropipette (p1000, p200)
- Filter tips(p1000, p200)
- 95% ethanol

Introduction

The **taco™** DNA/RNA Extraction Kit is designed for **taco™** Nucleic Acid Automatic Extraction System. Based on the magnetic separation technology, homogenized sample cells are lysed and nucleic acids are captured by silica coated magnetic beads. Washing Buffer is then applied to remove impurities, and Eluting Buffer to recover nucleic acids from magnetic beads following serial washing steps. This kit can extract virus DNA and RNA from shrimp muscle. Other sample types must be validated by users.

Note: For research use only. Not intended for any animal or human therapeutic or diagnostic use.

Intended Use

The **taco™** DNA/RNA Extraction Kit is intended to be used for extracting viral DNA and RNA from various sample types such as shrimp tissue. The **taco™** DNA/RNA Extraction Kit has to be used with the **taco™** Nucleic Acid Automatic Extraction System.

This product is intended to be used by professional users such as well-trained laboratory technicians familiar with molecular biology techniques.

Important Notes

- After receiving the kit, check the kit components for any damage. Contact GeneReach Biotechnology Corporation or your local distributor if the reagent bottles are damaged. Do not use damaged kit components since their use may lead to poor kit performance.
- Always change pipette tips between liquid transfers.
- When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.
- Discard gloves if they become contaminated.
- Do not combine components of different kits.
- Avoid microbial contamination of the kit reagents.
- To minimize the risk of infections from potentially infectious material, we recommend working under laminar air-flow until the samples are lysed.
- This kit should only be used by trained personnel.
- Disposal of waste must be compliant with local laws.

Nucleic Acids Extraction Procedure

A. Use of taco™ Sticker

For your convenience, user may put the taco™ Sticker on top of reagent bottles and/or on the rim of 96-Well Extraction Plate to avoid human error.

a. taco™ Sticker

- Plate Sticker:

  Apply the Sticker on the rim of 96-Well Extraction Plate.

- Bottle Sticker:

  Apply the Sticker on top of each reagent bottle.

b. Abbreviation Definition

LB	Lysis Buffer
M	Magnetic Bead
WA	Washing Buffer A
WAM	Washing Buffer A + Magnetic Bead
WB	Washing Buffer B
E	Eluting Buffer

B. Protocol

a. Load reagents into 96-Well Extraction Plate according to **Table 1** at the room temperature (16-30˚C) for the best kit performance.

Table 1. Loading Reagent

Step	Reagents
1	Add **200 µl 95% ethanol** to **column #1 (#7)**
2	Add **750 µl Washing Buffer A**[1] to **column #2 (#8)**
3	Add **750 µl Washing Buffer A** to **column #3 (#9)**
4	Add **750 µl Washing Buffer B**[2] to **column #4 (#10)**
5	Add **750 µl Washing Buffer B** to **column #5 (#11)**
6	Add **50 µl Eluting Buffer** to **column #6 (#12)**
7	Add **50 µl Magnetic Bead**[3] to **column #2 (#8)**

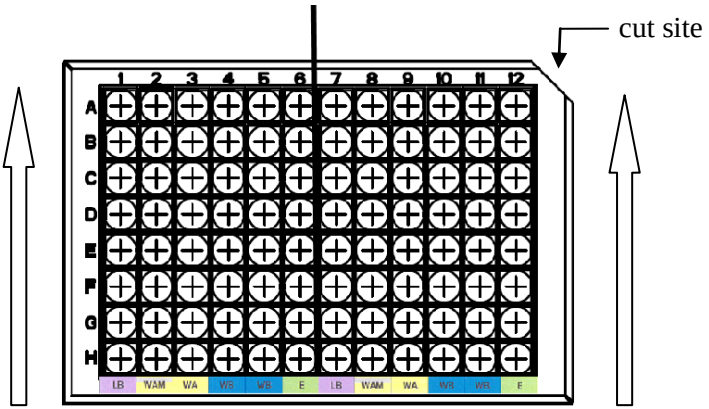
[1] **Ensure that 135 ml 95% ethanol has been added to Washing Buffer A before the first time use.**

[2] **Ensure that 230 ml 95% ethanol has been added to Washing Buffer B before the first time use.**

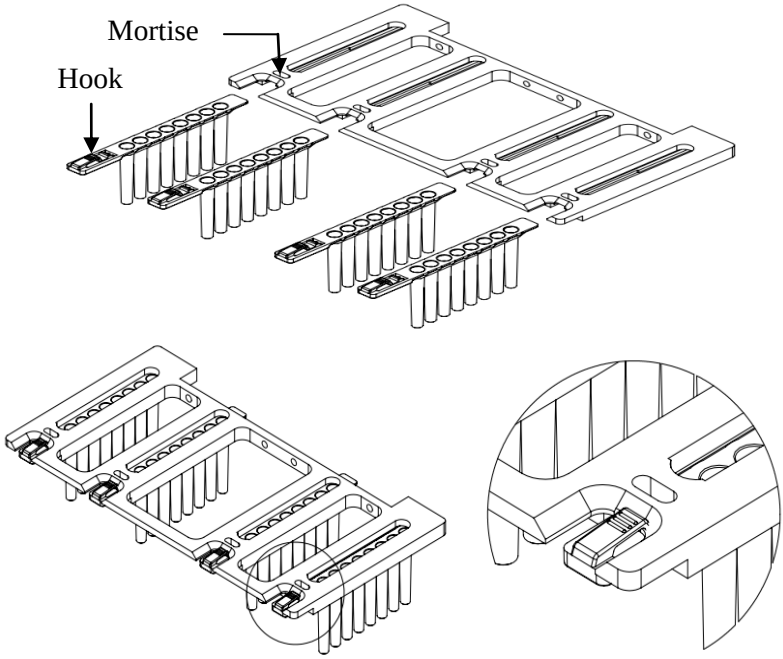
[3] **Magnetic Bead must be mixed until it's fully resuspended before each aliquot.**

b. Use micropipette (p1000) to load homogenized samples into column #1 and/or 7 (See "Sample Preparation", Appendix I).

c. Open the door of **taco**^{TM} and install 96-Well Extraction Plate with reagents and samples. Push 96-Well Extraction Plate completely to the bottom of plate holder. Ensure the cut site is located on the top right.



d. Install Mixing Sleeve and lift up the Hook of Mixing Sleeve to tenon the mortise (See the illustration below).



e. Press the door button of **taco™** to close the door and press "Start" button.

f. After extraction finished, **discard the Mixing Sleeves first**.

g. Take out the 96-Well Extraction Plate, then press "**Reset**" button.

h. Transfer the nucleic acids from column #6 and/or #12 to new micro-centrifuge tubes for use (See "Purity of Nucleic Acid", Appendix II).

i. It is strongly recommended to use freshly extracted nucleic acids for downstream applications such as amplification. Otherwise, the extracted nucleic acids should be kept at -80°C for longer storage (See "Storage of Nucleic Acid", Appendix II).

* Do not reuse the Plate & Sleeve.

Note: <u>Any deviation from the instruction may lead to a low yield of extracted nucleic acids.</u>

Product Use Limitations

The system performance has been validated using infected shrimp muscle for isolation of viral nucleic acids.

The kit is neither validated for use with bone marrow, cultured cells nor for whole blood, serum and plasma. The user is responsible for validating the performance of the **taco™** DNA/RNA Extraction Kit for any particular use.

The kit and plastic parts are not intended for any therapeutic or diagnostic of a disease for animals or humans.

Trouble Shooting

	Comments and suggestions
Low DNA/RNA yield	
(a) Magnetic Bead was not completely resuspended	Before starting the procedure, ensure that Magnetic Bead is fully resuspended. Vortex for at least 5 seconds before first use, and perform mild agitation before subsequent uses.
(b) Washing Buffer A and/ or B did not contain ethanol	Ensure the correct volume of ethanol is added to Washing Buffer A and/ or B; well seal the reagent bottles to prevent ethanol from evaporating. Repeat the extraction procedure with proper reagent (See "Protocol").
(c) Reagents were loaded in wrong order	Restart the loading procedure with a new 96-Well Extraction Plate. Ensure that all reagents were loaded in the correct order and wells. Repeat the extraction procedure with new samples.

	Comments and suggestions
(d) Poor sample quality	Using fresh sample for extraction is recommended. Poor sample quality may influence test result.
(e) Incorrect sample volume	Sample volume too high or low may influence the kit performance. User should optimize the sample quantity when dealing with different sample types.
(f) Mixing sleeve not installed	Contact your local distributor or GeneReach Biotechnology Corporation for assistance.
(g) Inappropriate operation environment	Operation temperature too high or low may lead to low yield of nucleic acids. Ensure the operation environment of **taco™** DNA/RNA Extraction Kit to be performed only under room temperature (16-30°C).
(h) Use non-recommended extraction instrument	User uses non-recommended instrument may influence the performance of **taco™** DNA/RNA Extraction Kit. We recommend user to apply it on **taco™**.

	Comments and suggestions
Poor DNA/RNA performance in downstream applications	
(a) Low volume of extracted DNA/RNA after the extraction is finished.	Repeat the extraction procedure with new sample using 100 µl Eluting Buffer.
(b) Insufficient DNA/RNA used in downstream application	Quantify the extracted DNA/RNA by spectrophotometer of the absorbance at 260 nm. (See "Quantification of Nucleic Acids", Appendix II)
(c) Excess DNA/RNA used in downstream application	Excess DNA/RNA can inhibit some enzymatic reactions. Quantify the extracted DNA/RNA by spectrophotometer of the absorbance at 260 nm. (See "Quantification of Nucleic Acids", Appendix II)

Comments and suggestions

A_{260}/A_{280} ratio of extracted DNA/RNA is low

(a) Absorbance reading at 320 nm was not subtracted from the absorbance readings at 260 nm and 280 nm	To correct for the presence of Magnetic Bead particles in the eluted solution, an absorbance reading at 320 nm should be taken and subtracted from the absorbance readings obtained at 260 nm and 280 nm.

Appendix I

Sample Preparation

- Animal Tissue: Shrimp muscle
  i. Grind the tissue (30 mg) with 500 μl Lysis buffer in 1.5 ml micro-centrifuge tube with disposable grinder.
  ii. Centrifuge at 12000 rpm for 5 minutes to remove the debris.
  iii. Transfer the supernatant 200 μl to column #1 (#7) of 96-WellExtraction Plate.

*The above sample preparation method is recommended for general muscle tissue containing high volume of protein; other sample types must be validated by users.

Appendix II

A. Storage of Nucleic Acids

Extracted Nucleic Acid should be stored at -80°C for storage.

B. Quantification of Nucleic Acids

The concentration of nucleic acids should be determined by measuring the absorbance at 260 nm in a spectrophotometer.

Use Eluting Buffer as the blank to calibrate spectrophotometer. If the purified nucleic acids need to be diluted before the quantification, the Eluting Buffer also has to be diluted first, and the same dilution factor needs to be applied for calculation.

Collect the absorbance reading of purified nucleic acids at 260 nm and 280 nm. The reading should fall between 0.1 and 1.0 to be accurate. An absorbance of 1 unit at 260 corresponds to 50 μg of nucleic acids per milliliter. The ratio between the absorbance values at 260 nm and 280 nm gives an estimation of nucleic acids purity (See "Purity of Nucleic Acids").

Carryover of Magnetic Bead may affect the A_{260} reading, but should not affect the performance of nucleic acids in downstream applications.

*Concentration of nucleic acids sample

= 50 μg/ ml × $(A_{260} - A_{320})$ × dilution factor

*Total amount of nucleic acids purified

= concentration × volume of sample in milliliters

C. Purity of Nucleic Acids

Purity is determined by calculating the ratio of corrected absorbance at 260 nm to corrected absorbance at 280 nm i.e., (A_{260}-A_{320}) / (A_{280}-A_{320}). A subtracted absorbance reading at 320 nm is to correct the presence of Magnetic Bead particles in the eluted solution. The purity of Nucleic Acid has an A_{260} / A_{280} ratio of 1.6~2.0.

Ⓒ2010 GeneReach Biotechnology Corporation. All rights reserved. For research use only. Not intended for any animal or human therapeutic or diagnostic use.